Chapter 4

Natural Antimicrobials, their Sources and Food Safety

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Additional information is available at the end of the chapter

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Abstract

With consumer awareness about food safety and quality, there is a high demand for the preservative (synthetic)-free foods and use of natural products as preservatives. Natural antimicrobials from different sources are used to preserve food from spoilage and pathogenic microorganisms. Plants (herbs and spices, fruits and vegetables, seeds and leaves) are the main source of antimicrobials and contain many essential oils that have preservation effect against different microorganisms. Mainly, herb and spices contain many essential oils and the examples include rosemary, sage, basil, oregano, thyme, cardamom, and clove. These essential oils are very effective against many pathogenic and spoilage microorganisms like *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter* spp., and *Staphylococcus aureus* and help to increase their quality and shelf stability. These antimicrobial compounds are also used in combination with edible food coatings and inhibit the ability of microorganisms to grow on the surface of food and food products.

Keywords: antimicrobial, essential oils, antimicrobial edible coatings

1. Introduction

Today, food safety is everybody's concern and it is very hard to find anyone who has not encountered an unpleasant moment of food-borne illness at least once in the past year. According to the report of WHO in 2005, there were about 1.8 million deaths caused by diarrhea (food-borne illness), and these diseases were due to the use of contaminated food and water [1]. The main cause of food-borne illnesses is the use of food contaminated by microbial pathogens, toxins, or radioactive components. When certain bacteria or pathogens contaminate food, they can cause food-borne illness or sometimes called "food poisoning." Food-borne illnesses are mild but sometimes they can even be deadly [2].

Food-borne pathogens (*Clostridium botulinum*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Bacillus cereus*, *Listeria monocytogenes*, *Cryptosporidium*, *Escherichia coli* O157:H7, etc.) are the main concern regarding the safety of food [3]. Food can contain microbiological pathogens that cause infections or intoxications, or chemical agents that cause acute or chronic intoxications. With special reference to meat and meat products, *Salmonella*, *E. coli*, *L. monocytogenes,* and *Campylobacter* are the main pathogenic organisms [4, 5].

There is an increase in the consumption of fresh food with the consumer demand for the ready-to-eat food and the desire to lead a healthy lifestyle. The challenges associated with the consumption of fresh food are short storage life and its association with food-borne diseases. To avoid these challenges, there is a commercial pressure of using chemical preservatives that prevent the growth of food spoilage agents, but the increase in the use of these chemical preservatives is negatively perceived by the consumer [6].

2. Antimicrobial agents and food safety

Traditional food preservation methods are less efficient in reducing the growth of food-borne pathogens in food products, and the ever-increasing demand for chemical-free food has paved the way for antimicrobials to be used in food industry [7]. The use of antimicrobials is a new technology by the food industry to increase the shelf life of food and overcome the issues of food quality and safety. These antimicrobials could be of natural or synthetic type, but natural antimicrobials are gaining much importance than synthetic ones. Even though synthetic preservatives are approved by government agencies for human use, many of these preservatives still threaten our health. Thus, researchers give more importance toward the potential of natural products for their antimicrobial activities [8–10].

3. Natural antimicrobial agents

Chemical compounds having pharmacological and biological activity and produced by living organisms are called natural products. Living organisms produce primary and secondary metabolites [11–13]. Primary metabolites are the products that have essential function in the organism, while secondary metabolites could simply be waste products or could have some important function in their producers. Secondary metabolites can be used as drugs against diseases such as cancer, inflammation (swelling), and so on and also have antimicrobial activity [1, 14]. Secondary metabolites possessing antimicrobial activity are called the natural antimicrobials and could be extracted from different sources like plants (fruits, vegetables, seeds, herb, and spices), animals (eggs, milk, and tissues), and microorganisms (fungi and bacteria) [15–17]. With special reference to plants, secondary metabolites are found to be healthy ingredients that work as antimicrobials or disease-controlling agents [4]. Owing to the potential of antimicrobials against pathogenic and spoilage microorganisms, these secondary metabolites gain much importance for the application in food products [18–20]. They contain the properties of antimicrobials and antioxidants at the same time and so are considered as a better option for food preservation as compared to synthetic preservatives [21].

Several researches have been conducted to find out the antimicrobial potential of natural products, especially the plant sources like fruits, vegetables, herbs, and spices because they are enriched with compounds having antimicrobial activity. Nowadays, there are more than 1350 plants with antimicrobial activities and more than 30,000 antimicrobial components have been extracted from plants [22]. However, many studies have also been conducted on antimicrobial potential of microorganisms and animals. Food applications of antimicrobials have also been investigated.

Nowadays, plant extracts and essential oils (EOs) have gained much importance due to their flavoring as well as antimicrobial potential [23]. Research conducted on the antimicrobial activity of the extracts from different fruit peels like banana, apple, pomegranate, sweet lime, orange, mango, and papaya indicated that fruit peel extracts have mild inhibitory effect against pathogenic bacteria [24–29]. Plants secondary metabolites contain many antimicrobial agents, so they have a greater inhibitory effect against Gram-positive and Gram-negative bacteria [14, 27, 30–32]. The chemical composition, concentration, and structure of the antimicrobial component determine their efficacy. Antimicrobial components of plant origin include flavonoids, thiosulfinates, glucosinolates, phenolics, organic acids, flavonoids, and saponins [31, 33, 34]. However, the main compounds with antimicrobial activity are phenols which include terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids [35–38].

Antimicrobial components in plant materials are commonly found in herbs and spices (rosemary, sage, basil, oregano, thyme, cardamom, and clove), fruits and vegetables (guava, pepper, cabbage, garlic, and onion, citrus), seeds and leaves (grape seeds, fennel, nutmeg, parsley, and olive leaves) [39–42].

In this chapter, we discuss the role of antimicrobials from different sources with special reference to meat and meat products. Consumption of meat is important for the growth, development, and maintenance of health in human beings. Meat is an animal origin food and is a rich source of proteins, vitamins, minerals, and so on which is why the safety of meat and meat products is of much importance [43, 44]. Proteins of meat are of much importance with a high amount of essential amino acids being available and of biological value. Meat and meat products are at a high risk of microbial spoilage and also cause losses to economy [45]. Although food industry has developed several new techniques for hygienic slaughtering and production of meat products, a major concern related to meat consumption is the presence of pathogenic microorganisms that cause food-borne diseases, for which raw meat provides an ideal substrate [46, 47]. *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes*, *E. coli*, and *S. aureus* are the most common meat spoilage agents that cause food-borne diseases worldwide [48]. Synthetic preservatives are used to overcome this problem, but their overuse leads to multidrug-resistant phenomenon in bacteria. Moreover, meat industry is facing a new trend of developing all natural food products, where there is no place for synthetic preservatives that could be the causative of food sensitivities, toxicities, and allergies [49–51].

Essential oils, as plant extracts possessing antimicrobial agents and also antioxidative and flavoring properties, can be considered as healthy ingredients to be used in meat and meat products. If essential oils are used in meat products, they can reduce the chances of food-borne diseases and can retard the oxidation of lipids in meat [52–54].

4. Antimicrobials from plant sources

4.1. Herbs and spices

Herbs and spices have long been used by human beings for different reasons like food additives, flavorings, and preservatives. They are considered the most commonly used natural antimicrobials against different pathogens. The antimicrobial activity of herbs and spices depends on the type of essential oil present in it, food type in which it has to be used, and the type of microorganism [11, 55–57].

The efficiency of essential oils from herbs and spices depends upon their chemical structure, in particular to the presence of hydrophilic functional groups such as hydroxyl groups [58]. Essential oils from clove, oregano, rosemary, thyme, sage, and vanillin are the most effective containing the phenolic groups [58]. They possess inhibitory activity against Gram-positive than Gram-negative bacteria [59, 60]. Essential oils have high vapor pressure and are able to reach pathogenic microorganism through gas or liquid phases. Many investigations have proved the antimicrobial efficiency of essential oils against several pathogenic and spoilage microflorae. However, the efficiency of essential oils depends upon the pH, storage temperature, and concentration of oxygen [61].

Some of the antimicrobial compounds that are present in spices and herbs are eugenol, thymol, thymol and carvacrol, vanillin, allicin, cinnamic aldehyde, and allyl isothiocyanate that are, respectively, present in cloves, thyme, oregano, vanilla, garlic, cinnamon, and mustard [26].

Essential oils possess antimicrobial activities against several pathogenic microorganisms present in meat, including both Gram-positive and Gram-negative bacteria [62]. Many studies have been conducted to analyze the effects of essential oils extracted from sources such as oregano, rosemary, thyme, basil, garlic, and clove, when used alone or in combination with other essential oils [4, 63].

Essential oils extracted from herbs and spices were found to be effective against several pathogenic microorganisms. Studies showed the antimicrobial activities of 14 essential oils (clove, oregano, rosemary, pepper, nutmeg, liquorice, turmeric, aniseed, cassia bark, fennel, prickly ash, round cardamom, dahurian angelipca root, and angelica) against four meat spoilage and pathogenic bacteria (*L. monocytogenes*, *E. coli*, *Pseudomonas fluorescens* and *Lactobacillus sake*), and the results showed that extracts of clove, rosemary, and cassia bark contained strong antimicrobial activity against these bacteria but a combination of rosemary and liquorice extracts was the best inhibitor against all four types of bacteria. Antimicrobials from herbs and spices are widely used by the industry, and government agencies have approved them to be safe [64, 65]. *Pseudomonas* bacteria are responsible for the unacceptability of meat sausages. The use of thymol extracted from thyme and oregano as an antimicrobial inhibits the growth of *Pseudomonas* in sausages [53]. Researches show that Marjoram, mustard, cinnamon, lemon grass, and rosemary extracts have inhibitory effects against *E. coli O157:H7*, *S. typhi*, and *Listeria* [66, 67].

The oregano essential oils have antibacterial activities against *E. coli*, *S. aureus*, *B. subtilis*, and *Saccharomyces cerevisiae*. The main component in the essential oil of oregano is carvacrol (80.5%). Some other studies show that *S. typhimurium* is more sensitive to oregano essential oils than *S. enteritidis* [68].

Sodium nitrite has been used as a preservative in meat and meat products, but researches showed that if it is used in combination with oregano essential oils, it will slow down the growth of bacteria more efficiently than sodium nitrite alone [14]. The amount of EOs used in meat and meat products should be higher than the dose used in in vitro conditions because of the interaction with components of meat. Antimicrobial essential oils can be used directly or as polyethylene oxide (PEO)-based antimicrobial packaging [69].

Another research showed that the addition of oregano essential oils at a concentration of 0.7% will provide antimicrobial activity in minced sheep meat against *S. enteritidis*. In vitro tests detected the antibacterial activity of oregano oil against *S. enteritidis* in foods such as traditional salted fish and cod fish [70].

Mustard and horseradish essential oils also have antimicrobial activities against Gram-negative bacteria. Major antimicrobial agent in both is allyl-isothiocyanate [71–73].

When applying the antimicrobials in meat or meat products, depending upon the properties and type of pathogen, some EOs are more effective than others. Eugenol, coriander, clove, oregano, and thyme oils were found to be effective at levels of 5–20 μl/g in inhibiting *L. monocytogenes* in meat products, while mustard, mint, and sage oils were less effective or ineffective [74].

Rosmarinus officinalis L. commonly called rosemary is cultivated in southern Europe and is used as a flavoring agent due to its better flavor, high antioxidant, and antibacterial capacity [74, 75]. Carnosic acid and carnosol are the major antimicrobial components of rosemary and are effective against both Gram-negative and Gram-positive bacteria. In meat and meat products, rosemary oil has high antibacterial activity against *L. monocytogenes* [76].

Thyme essential oils have high antimicrobial activity owing to the presence of different compounds. The most prominent of all identified compounds of thyme essential oils were thymol (50%), followed by p-cymene (24%), linalool (4.6%), γ-terpinene (4.1%), and 1,8-cineole (4.3%). Thyme oils are effective against *L. monocytogenes* at a dose level of 5–20 μl/g. When added at a dose level of 0.3–0.9%, they are very effective against *E. coli* in beef. In vitro antimicrobial activity of thyme essential oil has been tested against *E. coli* at a temperature higher than that of refrigeration [77, 78].

Extensive research has been conducted to analyze the efficiency of essential oils against *Salmonella*, and results showed that oils extracted from thyme and oregano reduce the growth of *Salmonella* up to many folds of colony-forming unit (CFU) levels, while cinnamon oils at a rate of 7000 mg/kg of meat have strong antibacterial activity against *Salmonella* [62, 77].

Research has been conducted to find out the antimicrobial activity of clove oil against *L. monocytogenes* in minced mutton. Thymol essential oil from thyme at a concentration of

250–750 mg is used in fresh minced beef in combination with modified atmosphere packaging against different microorganisms and also increases the shelf life of beef [26, 29].

Sage essential oil is used at a concentration of 0.3% in minced beef in combination with soy protein. Rosemary or Chinese mahogany (500, 1000, and 1500 ppm) is used to increase fresh chicken sausage [14, 69, 79].

4.1.1. Safety aspect of essential oils

Antimicrobial agents, though very effective against microbial population and able to extend the quality and shelf life of meat and meat products, should be added with care because they can cause some side effects. Many essential oils like thymol and eugenol can cause mucous membrane irritation, if used in higher concentrations. In vitro studies of various essential oils like carvacrol, carvone, thymol, and so on show a mild to moderate toxic effects [30]. Some essential oils can cause allergy or some can have photoactive molecules which can cause phototoxic reactions [80, 81].

4.2. Fruits and vegetables

Many fruits and vegetables are nowadays well known to have antimicrobial effect against different pathogenic and spoilage microbes due to their contents of phenolic and organic acids. Fruit peels that are mostly discarded also contain antimicrobial compounds [30, 82].

Research showed that the antimicrobial activity of orange peel and capsicum was due to the presence of phenolic compound (coumaric acid) [83]. In minced beef, the extracts of capsicum annum have inhibitory effect against *S. typhimurium* and *Pseudomonas*. The minimum dose level of capsicum extract was 1.5 ml/100 g of minced beef to inhibit the growth of *S. typhimurium*, while a dose of 3 ml/100 g was required for a bactericidal effect against *P. aeruginosa* [83].

Pomegranate extract reduces the growth of *E. coli*. The peel of pomegranate contains different phenols and flavonoids that have great antimicrobial activity against Gram-positive bacteria. Peel extracts have inhibitory effects against *S. aureus* and *B. cereus* at a concentration of 0.01%. The addition of pomegranate peel extract to chicken meat products increases its shelf stability by 2–3 weeks during chilled storage and its extract is also effective in controlling oxidative rancidity in these chicken products [7, 62].

Citrus peel extract, lemon grass, and lime peel extracts were investigated for their antimicrobial activities in meat and meat products. The extracts showed high potential of antibacterial activity against *B. cereus*, *S. typhimurium*, and *S. aureus*. Hot water extract of lemon fruit peels, seeds, and juices displayed promising evidence of antibacterial activity against bacteria *E. coli*, *P. aeruginosa*, and *S. aureus* [84, 85].

Garlic is a potential inhibitor for food pathogens. Foods contaminated with pathogens pose a potential danger to the consumer's health. The use of garlic can increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods. Garlic extract has antimicrobial activity due to the presence of an organic sulfur compound allicin, which acts

as a growth inhibitor for both Gram-positive and Gram-negative bacteria including *E. coli*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus*, *and Helicobacter pylori*. Garlic aqueous extract has antibacterial properties against *S. aureus* present in hamburger. Freshly ground garlic in combination with lean camel meat at a concentration of 10, 15, and 25% was used to increase the shelf life of meat at different temperatures (rooms, incubators, refrigerators). After 4 days of storage at room temperature, 12 days of incubation, and 28 days of refrigeration, it was found that treatments with 15 and 25% garlic resulted in complete inhibition of microbial growth with no sign of any organoleptic spoilage of the meat [11].

The antimicrobial effect of onion extract on the fresh beef fillet meat was investigated. Beef fillet samples were cut into pieces and treated with 5, 10, 20, and 50% onion-water extract (v/v) and stored in refrigeration conditions at 4°C. Microbiological quality of the samples was investigated during storage for 9 days. Increasing concentrations of onion extract significantly affected *E. coli* and yeast-mold counts, but *Pseudomonas* spp., aerobic mesophilic bacteria, and total coli forms were not affected significantly for some concentrations and days.

Antimicrobial efficacy of curcumin, one of the active components of the *Curcuma longa* (turmeric) plant, was evaluated against food pathogens in a minced meat medium. *S. typhimurium*, *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus* strains were used as food pathogens [86].

5. Antimicrobial edible coatings

Today, many fresh products are available commercially with best nutritional profile and low cost of production. Consumers also prefer consuming fresh meat and meat products, but a limit for the commercial availability of fresh meat is its low storage life because of high moisture contents that cause the growth of pathogenic and spoilage microorganisms [87].

To avoid this, the spoilage use of antimicrobials is one of the best ways to increase the shelf life of these perishable food products especially meat and meat products. The use of antimicrobial films and coatings dates back to twelfth century. The only difference between film and coating is its thickness. There are many ways of applying these antimicrobials on food products to enhance the natural appearance and safety of fresh meat and meat products like spray or spread of antimicrobials on meat [88, 89].

By the combination of different preservation techniques, researchers have been successful in achieving the objectives related to microbial quality storage life of perishable products. The addition of natural antimicrobials in combination with modified atmosphere packaging and refrigeration has proven to show the best results. Antimicrobials can also be added in coatings and films to be used in meat and meat products [88, 90].

The use of antimicrobials in edible films and coatings is an emergent technique that is helpful in enhancing the quality and safety aspect of food. This technique includes a control release of antimicrobial agents in effective concentration in the food product, when required.

The use of oregano essential oil (EO) as natural antimicrobial in combination with modified atmosphere packaging and refrigeration highly enhances the storage life of fresh beef

or chicken during storage. Whey protein isolate coatings containing antimicrobial agents like oregano EO, 3-polylysine, or sodium lactate were used on fresh beef under refrigeration, which was evaluated against the progression of microflora like *Pseudomonas* bacteria [91]. By using 1.5% of oregano EO or 0.75% of 3-poly-lysine, the growth of *Pseudomonas* spp. was reduced and the development of lactic acid bacteria was completely inhibited. Both *Pseudomonas* spp. and total viable microorganisms were completely inhibited with 2% sodium lactate, even though the effect on LAB was less intense [89].

The effect of soy protein isolate films containing up to 5% of oregano and/or thyme EO was evaluated to be effective against coliform and *Pseudomonas* spp., but not significantly effective against total viable microorganisms, LAB, or *Staphylococcus* spp. in vacuum-packaged minced beef burgers for a 12-day period of cold storage at 4°C. Carvacol and cinnamon aldehydes, the main active compounds of oregano and cinnamon essential oils, were evaluated for their antimicrobial activity; they were incorporated in edible films based on apple puree containing 1.5 and 3% of carvacrol or cinnamaldehyde over chicken breast under refrigeration. These films inactivated the autochthonous spoilage microflora of chicken [89].

Whey protein isolates-based edible films were evaluated for antimicrobial activities with different essential oils. These films showed high effectiveness against *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica Typhimurium*, when used in combination with 1% sorbic acid in meat sausages. Oregano containing carvacrol as antimicrobial agent and clove containing eugenol EOs were highly effective against *S. aureus*, *Salmonella*, and *L. innocula*. Coatings act as barrier against oxygen transfer leading to growth inhibition of aerobic bacteria. Chitosan has been used as an antimicrobial agent and also as a coating and wrapper in salami and film and coating combined with lauric arginate and nisin to reduce *L. monocytogenes* population in sliced turkey deli meat and also in seafood and fish [63, 64].

Similarly, milk protein coatings are used in beef in combination with oregano essential oils against *E. coli* and *Pseudomonas*. Chistosan coatings dissolved in lactic acid in combination with 1% acetic acid are used in roasted beef products against *L. monocytogenes*. Similarly, chitosan coatings in combination with oregano oil at a concentration of 0.7% are used against *Pseudomonas* spp. and *Brochothrix thermosphacta* [91]. Gelatin films are used in Turkey bologna in combination with nisaplin-based films (GNF) (0.025–0.5%; w/v nisin) against *L. monocytogenes* bacteria [89].

Chitosan coatings in different molecular weights and viscosities (14, 57, or 360 mPa) were used in Atlantic cod fish against psychotropic bacteria. Whey protein coatings were used in smoked fish in combination with Lactoperoxidase system (0–0.5%, w/v) against *L. monocytogenes*. Gelatin films were used in sardine pilchardus in combination with oregano extracts against Enterobacter bacteria. Alginate, carrageenan, pectin, gelatin, or starch coatings were also used in smoked salmons in combination with sodium lactate against a mixture of *L. monocytogenes* [92].

6. Conclusion

All the researches and studies conducted till now have proved that the use of synthetic preservatives to increase the shelf life of food and food products is in any way harmful for the

human health, so there is a call for the use of natural products as preservatives to increase the quality and shelf stability of the food and food products. Natural antimicrobials contain all the qualities to be used as preservatives especially in meat and meat products, and plants are the main source of these antimicrobials.

Plant essential oils have great antimicrobial activity against Gram-positive and Gram-negative bacteria owing to the potential of phenolic compounds. Essential oils from herbs and spices like clove, oregano, rosemary, thyme, sage, and vanillin are the most effective against spoilage and pathogenic microorganisms like *L. monocytogenes*, *E. coli*, *P. fluorescens*, *L. sake*, *S. aureus*, and *B. subtilis*. Mustard and horseradish essential oils also have antimicrobial activities against Gram-negative bacteria. Major antimicrobial agent in both is allyl-isothiocyanate. Antimicrobial agents, though very effective as antimicrobial agents, should be used with care because they can cause side effects like irritation. Many fruits and vegetables also contain antimicrobial activity against pathogenic and spoilage microbes. Extracts of capsicum annum showed antimicrobial effects against *S. typhimurium* in minced beef; similarly, pomegranate extracts reduced the growth of *E. coli*. Citrus peel extract, lemon grass, and lime peel extracts showed great antimicrobial effect against *B. cereus*, *S. typhimurium*, and *S. aureus*. Garlic is a potential inhibitor for food pathogens. Garlic aqueous extract has antibacterial properties against *S. aureus* present in hamburger.

To increase the shelf life of meat and meat products, a new trend is the use of antimicrobial in edible films and coatings in combination with different packaging techniques. Oregano essential oils in combination with modified atmosphere packaging highly increase the shelf life of chicken and beef. Whey protein isolate coatings added with oregano essential oils in combination with refrigeration were very effective against *Pseudomonas* in beef and beef products. Whey protein isolate-based edible films were evaluated for antimicrobial activities with different essential oils and were very effective against *S. aureus*, *Salmonella*, and *L. innocula*. Antimicrobials can be sprayed upon meat and meat products or meat can be dipped into them. They are completely harmless to human health owing to the potential of all natural compounds, so there is an increasing market for the natural antimicrobials to be used as preservatives.

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